

## TOXOPLASMA STRAINS

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The inventors have generated multiple gene knockout strains of *T. gondii*, listed below.

### **RHΔ $hx\Delta ku80$ TIR1 (RHΔ $ku80\Delta hxgprt$ /pTub 5'UTR-OsTir1-3xFlag-DHFR 3'UTR-CAT):**

The inventors used the CRISPR/Cas9 system for genome editing, combined with the AID degradation system to generate the *Toxoplasma gondii* strain. It was used to study plasma membrane association and PKG function in *Toxoplasma gondii*.

**Publication:** [Plasma Membrane Association by N-Acylation Governs PKG Function in \*Toxoplasma gondii\*](#)

### **DiCre KB1 (RHΔ $ku80\Delta hxgprt$ /DiCre):**

To generate a DiCre recipient strain expressing both subunits in the same genomic locus, p5RT70DiCre-HX was transfected into RH  $hxgprt^-$  (RH  $hxgprt^-/diCre$ , referred to here as RH DiCre). Expression of Cre recombinase subunits was confirmed by western blot analysis with antibodies to FKBP12 and FRB.

The  $ku80\Delta diCre$  recipient strain was generated by replacing HX with  $diCre$  in the  $ku80\Delta HX$  strain by homologous recombination ( $ku80\Delta HX\Delta diCre$ , referred to here as  $ku80\Delta diCre$ ). The 5' UTR-DiCre- $ku80$  3' UTR cassette was transfected into  $ku80\Delta HX$  strain, and subsequently  $ku80\Delta diCre$  parasites were selected using 6-thioxanthine to remove HX. Integration of  $diCre$  into the  $ku80$  locus was confirmed by analytical PCR on genomic DNA using  $ku80\Delta HX$  fw (1) and  $ku80$  rv (1') primer pair to check for the presence of  $hx$  in the  $ku80$  locus.

**Publication:** [Conditional genome engineering in \*Toxoplasma gondii\* uncovers alternative invasion mechanisms](#)

### **Pru Δ $ku80$ SL1 (PruΔ $ku80\Delta hxgprt$ clone SL1):**

The inventors used electroporation to transfect the parental Pru (PruΔ $ku80\Delta hxgprt$ ) *T. gondii* strain. All transfected plasmids were linearized 5' of the 5' target DNA flank prior to transfection using unique restriction enzyme sites designed into the targeting plasmids. Forward selections to integrate the pmini-HXGPRT selectable marker were performed in mycophenolic acid and xanthine. Negative selections to excise HXGPRT were performed in 6-thioxanthine. Negative selections using the cytosine deaminase (CD) selectable marker were performed in 5-fluorocytosine. Negative selections to delete UPRT were performed in 5-fluorodeoxyuridine (FUDR). After transfection, parasites were allowed to replicate for 24 h without selection to allow replication and ramp up homologous recombination, and then selections were launched and continuously maintained through verification steps of cloned isolates

**Publication:** [Type II \*Toxoplasma gondii\* KU80 Knockout Strains Enable Functional Analysis of Genes Required for Cyst Development and Latent Infection](#)

### **ME49 TIR1 (ME49Δ $ku80::Luciferase\Delta hxgprt$ /pTub 5'UTR-OsTir1-3xFlag-DHFR 3'UTR-CAT)**

**ME49 $\Delta$ hx $\Delta$ ku80 SL1 (ME49 $\Delta$ ku80::Luciferase $\Delta$ hxgprt clone SL1)**